COACERVATION OF STARCH

by

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INTRODUCTION

The name "coacervation" has been given to the phenomenon of phase separation of colloidal material in which a colloid-rich layer is separated out of the system in a certain range of concentrations when a micro- or macro-molecule is added to the colloid. In most cases such a layer, as it is separated out, is a mass of spherical droplets or myelin structures, or sometimes appears as a relatively large sheet that may be visible under the microscope. In early work on this phenomenon, Bungenberg de Jong and Kruyt (4,5) have given the names: coacervate to the layer rich in colloids, equilibrium liquid to the layer poor in colloids and coacervation to this phenomenon of phase separation.

The most frequently demonstrated coacervate has been a microscopic droplet produced by adding a salt such as NaCl or Na_2SO_4, or a simple phenol to a gelatin solution at a certain pH (6,8,11). As none of these cause ionic neutralization, it is explained that salts or phenols, by forming hydrogen bonds with water molecules, act as coacervating agents through the reduction of the activity of water, i.e., these electrolytes take up water of ionic hydration which is surrounding the colloidal particle of gelatin.

As the result, unstabilized colloidal particles coalesce to form microscopic droplets which contain gelatin, salts or phenols and water as their constituents. Alcohol may also replace the salt in this instance, in which case the coacervate droplets contain alcohol together with gelatin and water (12,21,38). This is termed a simple coacervation

in which the coacervation of a colloidal sol is not due to charge effect but simply due to desolvation by other substances, either micromolecular or macromolecular, as mentioned above.

The term complex coacervation (13) is given to the phenomenon when the desolvation giving rise to a coacervate is due to opposition of charges of the respective constituents. Thus highly desolvated coacervates are formed by mixing two oppositely charged hydrophilic sols. A typical example of complex coacervation is that which occurs upon the combination of gum arabic and gelatin, which has been observed by many workers (7,9,10). In every case, adequate opposition of charge between tha two colloids has been essential.

Both simple and complex coacervation are reversible. This is best demonstrated by adding small amounts of HCl and NaOH alternately to the complex coacervate of a gelatin and gum arabic mixture so that the pH of the mixture may change to below and above pH 4.8 (pI of gelatin). The coacervate forms when the pH is below 4.8 and disappears when the pH is above 4.8 (37,55). These changes can be repeated until a sufficient amount of salt is accumulated, when the considerably high concentration of salt hinders the formation of a coacervate. It is in fact characteristic of complex coacervation that the phenomenon is suppressed by the addition of a high level of an indifferent salt (23). Reversibility can also be demonstrated by elevating the temperature of the system after a coacervate is formed. In this instance the coacervate disappears but it reappears when low temperature is reattained (5).

Several instances of starch coacervation have been demonstrated,

though there is the adverse suggestion that starch fails to form a coacervate (39). This is attributed to the weak charge which starch molecules generally show when dispersed in water. The actions of freezing, alcohol or chloral hydrate upon starch paste, however, provide interesting modes of formation of starch coacervates (40,42,49,54,60). Starch sponge produced by freezing has been suggested as a useful industrial or medical material (3). It has been suggested that the failure of the thickening effect of starch in food manufacturing processes and the deterioration of starch paste in sizing may sometimes result from starch coacervation.

Starch coacervation as related to freezing, or addition of alcohol, salt or chloral hydrate has received preliminary study in this department. This thesis presents further experimental results of the continued work on starch coacervation. Particular attention has been focused on the use of chloral hydrate and gelatin in starch coacervation, the characteristics of such coacervates and the possible mechanism involved in their formation.

REVIEW OF LITERATURE

Separation of a colloid into two distinct layers by adding certain electrolytes has long been known. In this instance the term coagulation, which is the most commonly known phase change in colloidal solution, was used in most of the old literature. Tiebackx (55) in 1911, observed that a mixture of equal amounts of dilute solutions of gelatin and gum arabic coagulated when a small amount of HCl or H_2SO_4 was added. Hevesy (35) showed evidence that the electrically charged microparticles were capable of combining with water molecules, and later Mines (43) indicated that a complex trivalent cation had greater precipitating power than a monovalent cation for a lyophobic colloid. A large number of ionized colloids which were capable of forming coacervates were extensively studied by Bungenberg de Jong and his coworkers. They explained the phenomenon in these instances as being principally based upon the mutual interaction of the ions (5). For example, 3% aqueous solutions of gum arabic and gelatin which were adjusted to pH 3.5 with HCl were mixed in equal amounts at 35°C. White cloudiness immediately appeared and settled to the bottom of the container after prolonged standing at 35°C. White cloudiness was described to be microscopic droplets which contained 12% solid substance while the supernatant liquid contained less than 0.1% of the total solid. Bungenberg de Jong named this phenomenon "coacervation". He stated that the coacervate in this instance was in a low dispersed state, whereas in the common colloidal coagulation, the coagulate was in a highly dispersed state. The force that led the colloidal particles to unite with one another was attributed to the desolvation of the water coating of the colloidal particles. He suggested

that the stability of a colloidal sol results from two factors: the solvation of the particles and their surface potential. Upon lowering of the surface potential, by addition of either electrolytes or oppositely charged colloid, the original colloid would be desolvated and precipitated out. Total desolvation would produce coagulation and partial desolvation would produce coacervation. It was found that a lower amount of a high valence ion was required to induce coacervation than of a low valence ion (14,15). The diameter of coacervate droplets was variable, depending on the conditions under which the coacervation was induced, i.e., concentration of each constituent, temperature, pH value of the solution, etc. (16,18).

Dervichian (24) summarized coacervation as a phase separation. He pointed out that a certain degree of desolvation preceded the appearance of a coacervate. Therefore coacervation involves separation of a new phase (coacervate) in which the constituents of the original colloidal system are included in new proportions, depending upon the degree of hydration. He further pointed out that use of the term coacervate for a complex is, therefore, incorrect.

It was observed that the viscosity of a colloid decreases when the coacervate starts forming (20). This led Bungenberg de Jong to speculate that the force which unites colloidal particles is intermolecular association due to decreased solubility of a colloidal molecule in the extramolecular fluid because of highly increased salt concentration. This sudden reduction of the amount of occlusion-liquid inside the colloidal particle, -- rather than desolvation of an extramolecular solvation coating, -- causes the observed viscosity decrease (9).

Basu and Bhattacharya (2) discussed coacervation in relation to the alternative configurations of large molecules. They suggested that a molecule or a polymeric chain which is in extended configuration in the solvent will give a solid product when it is separated out of solution, whereas a coacervate will be obtained if the molecule is in the coiled state in the solvent because large numbers of solvent molecules are trapped and immobilized inside the coiled structure. They showed experimentally that without NaCl, gelatin separated out of solution as a granular solid at both high and low pH, when the solution was mixed with alcohol. With NaCl, coacervation took place upon addition of alcohol because NaCl considerably suppresses the electric charge of dissociated gelatin molecules due to the common-ion effect; the resulting less extended molecular configuration is favorable to coacervate production. When gum arabic was substituted for the gelatin, same result was obtained. It is evident from this experiment that in coacervation with colloidal particles of both signs, some other kind of repulsive force must be present; solvation being the most reasonable explanation (24). Theoretical confirmation has been given by Langmuir (41).

Vacuolization of a coacervate drop is reportedly a very general phenomenon in which the liquid in the coacervate diffuses out of the colloid molecule, without disturbing the tiny droplets of coacervate, to form vacuole inclusions (5). This phenomenon is often compared with that of formation of vacuoles in protoplasm in the cell of biological tissue (6,19). Bungenberg de Jong (17) showed that mixture of gelatin and gum arabic with the action of yeast produced a coacervate which was morphologically similar to that of a plant cell at a specific condition.

Dobry (25) in 1938 found that unionized colloids can undergo coacervation. Thus cellulose acetate in a mixture of alcohol and tetrachloroethane or chloroform forms a coacervate without evidence of any charge effect (27). In this instance, the osmotic pressure of both coacervate and equilibrium liquid were found to be equal and almost nil (26,28,29). Dobry concluded that coacervated colloids are not diffusible, therefore they exert no osmotic pressure on the cell membrane. This gave rise to the interesting concept that in nature coacervation might exist in the cell structure of biological tissues, i.e., coacervates of different nature could exist side by side (in the cell) without disturbance of osmotic pressure. She further suggested that coacervation allows nature to store substances necessary for active metabolism in perfect liquid form, in a state of physicochemical equilibrium. Thus coexistance of protein and carbohydrate at one spot in aquatic unicellular organisms is largely dependent on coacervation and, in her opinion, it constitutes the necessary condition for the formation of the simplest organisms (30).

Oparin et al (44,45,48) studied coacervates made from various biological body proteins and carbohydrates with the participation of enzymes (α-amylase and a bacterial catalase). They found that enzymes are capable of forming coacervates with systems containing proteins and carbohydrates. Thus a coacervate droplet was produced by mixing glucose-1-phosphate, phosphorylase (obtained from a potato slice) and gum arabic-histone in acetate buffer at pH 6. Starch was produced in the droplet when it was held at a considerably elevated temperature. The authors postulated coacervate drops to be "initial systems on the way to the

origin of life" (46,47).

Some coacervates of phospholipids, phenolic compounds and pigments have been found in plant cells which lack some nutrients (1,32,51). Typical of these coacervates, reported by Reed and Duefrenoy (50) was a refringent colloid which occurred as bodies in vacuoles of cells affected by virus fungi or in zinc deficient orange leaves, buds and root tissue. Sufficient supplementation of the lacking micro-element was found to prevent formation of these coacervates.

Under certain conditions starch and its linear and non-linear components undergo coacervation. Ostwald and Hertel (49) reported the separation of immiscible layers from mixtures of gelatin and various starches with or without salts over a considerably wide range of pH. As he failed to point out the morphological characteristics of the products, it remains unknown whether the immiscible layers which he obtained were coacervates or solid precipitates such as was later described by Basu and Bhattacharya (2).

Koets (39) in 1935 stated that amylose often fails to form coacervates, principally because of the extreme smallness of ionic charge which amylose exhibits in aqueous solution. However, the character of amylose can be completely changed in this respect by the introduction of some other groups which exhibit strong ionic charges. Thus Koets successfully obtained a coacervate of amylophosphoric acid and protein (40). Since amylopectin is responsible for the formation of starch paste and exhibits high viscosity which does not follow Poiseulle's law (indicating the presence of micelle conglomerates or gel fragments in solution), it was further postulated by Koets that the amylopectin might be

possibly of the nature of a coacervate of amylophosphoric acid and one or more proteins.

An interesting instance of coacervation of starch, induced by freezing, was first reported by Scharling according to Bice _et al_ (3), long before the term coacervation was coined. This was eventually recognized as being a case of coacervation and studied as such by MacMasters _et al_ (42,54).

Upon slow freezing (at -2 to -3°C) of starch paste (or amylose or amylopectin paste) at from 0.1 to 10% concentration, a spongy coacervate is obtained. The phenomenon was described as being a partial desolvation of starch sol as the paste gradually freezes. Morphological characteristics were reported to vary depending on the experimental conditions and ranging from droplets or myelin structures to large sheet forms with some myelin projections. Usefulness of such a spongy starch coacervate as a surgical agent was suggested by Bice _et al_ (3), and its use in confections has also been demonstrated by Hilbert _et al_ (36). The material is on the market as a hemostatic agent; this property was discovered by Rosenfeld (52). The coacervates formed by starch with ethanol or chloral-hydrate have many similarities to the amylose complex formed with butanol (53). MacMasters _et al_ suggested that the amylose complex formed with fractionating agents, such as butanol or other alcohols, nitroparaffin or chloral hydrate, might originally represent a specific case of coacervation of the linear component of starch with subsequent crystallization accompanied by loss of liquid. Thus a coacervate obtained by mixing starch paste and ethanol was reported to have an iodine-sorption value of 131 mg iodine per gram dry substance which would indicate that two-

thirds of the carbohydrate present was amylose. Chloral hydrate-starch coacervate was found to be more elastic and birefringent. Iodine-sorption values and X-ray diffraction analysis indicate that both ethanol and chloral hydrate are of great value as a fractionating agent to separate amylose from amylopectin.

Doi and Nikuni (31) found that starch, amylose or amylopectin can be quantitatively recovered in granule form from a hot colloidal sol by adding a large amount of gelatin. Although X-ray diffraction indicated relatively poor crystallinity in such recrystallized granules, it provides the evidence that granulation (or partial crystallization) may take place as the water is progressively removed from a starch coacervate. From this evidence it is possible to speculate as to the existence of some similarity between coacervation of starch by freezing and by the action of gelatin. MacMasters _et al_ (42) discussed the possible mode of starch granule formation in plant cells, principally based upon the fact that corn starch granules exhibit no birefringence until their moisture content is lowered, either artificially or in the natural process of kernel maturation. This was also observed by Evans (33). At the very beginning of starch deposition, liquid globules of carbohydrate material contain very tiny starch granules. This is described to be very similar to that of the coacervate-within-a-coacervate system often observed after freezing starch pastes and gels. It was further described that the hilum of the starch granule is a space containing no starch, which, in dried granules, is filled with air. This may be the equivalent of a vacuole of the original coacervate from which the liquid has disappeared during drying. The work of Oparin _et al_ as previously

described -- in which starch is synthesized by the action of enzymes within a coacervate droplet -- seems to give support to this theory.

Recently, a useful type of application of coacervation has been reported and the processes have been patented by industrial firms such as Upjohn Co. (56,57,58). The basic process was described as useful for the production of a coacervate which consisted of a stable complex of an oil emulsion with a colloidal sol (example, a sol of gelatin in H_2O) where oil drops were encapsulated by the sol. The encapsulated oil consisted of a hydrophilic liquid-in-oil emulsion, in which the dispersed hydrophilic phase contained the material of interest. Thus urea fertilizer was encapsulated with gelatin sol for prolonged nitrogen release when applied in the field; cod liver oil or castor oil was encapsulated with gelatin sol for pharmaceutical purposes. Other applications, such as coating honey-in-oil emulsion for food uses, encapsulation of photographic developer for slow release, and coated Fe powder for use in a magnetic memory device, were reported (59).

MATERIALS AND METHODS

Materials

A. Starch - About 10 pounds of Hard Red Winter wheat flour (Lot No. 364-24) obtained from milling in the pilot mill of the Department of Flour and Feed Milling Industry, Kansas State University, was used as a starch source. Wheat starch was separated from the flour by the gluten washing method. The flour was made into a stiff dough with distilled water and allowed to stand for 2 hours in the refrigerator for the gluten to "develop". The flour dough ball was kneaded under a stream of distilled water and the impure starch slurry that passed through a No. 11XX nylon sieve was centrifuged at 580 x g. The "tailings" fraction appearing as a layer above the starch was carefully eliminated by scraping and the starch was collected, resuspended in distilled water, recentrifuged and again separated from any trace of tailings. After 3 such separations, the prime starch was air dried in a well ventilated room at 25°C for 24 hours, then oven-dried at 35-38°C for 48 hours. Clumps of starch were broken by hand in order to prevent them from forming a hard mass. This starch contained 8.70% moisture, 0.09% ash, 0.40% ether extract and 0.19% protein. The starch was further treated with 85% methanol by refluxing it on a water bath for 6 hours (Two hours each for 3 times. After each 2 hours, methanol was removed by vacuum filtration and fresh methanol was added for another refluxing). This defatted starch was used for all experiments.

B. Amylomaize - Amylomaize I, a product of American Maize Products Company, Roby, Indiana, containing 56-57% amylose and previously

defatted with 85% methanol was used.

C. Amylopectin - A product of Nutritional Biochemicals Corp., Cleveland, Ohio, was used.

D. Chloral hydrate - $C_2H_3Cl_3O_2$, U.S.P. grade, manufactured by Fisher Scientific Company was used.

E. Gelatin - A granular gelatin (for bacteriological use) manufactured by Fisher Scientific Company was used.

Methods

A. Coacervation of starch by the action of chloral hydrate. - Various amounts of defatted wheat starch, amylomaize or amylopectin were suspended in test tubes which contained a 6 M (ca. 100 w/v%) chloral hydrate solution to make a final starch concentration of 1 to 10%, in 1% steps. They were heated in a boiling water bath for 30 minutes. A solution of over 5% starch, amylomaize or amylopectin was found to be extremely viscous and to tend to solidify when being cooled. Therefore no more than 5% concentration was used in later experiments.

Microscopic examination was made to confirm that complete gelatinization was reached. It was found that the starch, amylomaize or amylopectin in the above range of concentration might be completely gelatinized or (in case of the fractions) dispersed in 24 hours without heating. The resulting solutions were viscous, transparent liquids which upon prolonged standing (more than one month) at room temperature did not acquire any cloudiness or appearance of suspension.

The dilution of each of these liquids with water at specific pH was made in 1:2, 1:4, 1:6, 1:8, 1:10 and 1:20 ratios. The pH of the

water was adjusted with 1N HCl or NaOH to 3, 4, 5, 6, 7 or 8. The dilution was carried out at both room temperature and 40°C. These experiments were conducted by using amylomaize, amylopectin or defatted wheat starch in the above mentioned procedure.

Separate experiments were conducted with 4% of defatted wheat starch, amylomaize or amylopectin in chloral hydrate of various concentrations, such as 5, 10, 20, 30, 40, 50 and 70% (w/v).

B. Coacervation of starch by action of chloral hydrate, gelatin and salts. - The solution of starch or amylomaize or amylopectin in chloral hydrate was mixed with gelatin in a test tube at various proportions and temperatures. A solution of 4% starch, amylomaize or amylopectin in 6 M chloral hydrate solution was used in most casea because it was found most convenient for this experiment. The concentration of gelatin was varied from 3 to 10%, however a concentration of higher than 6% tended to solidify at room temperature, therefore 3 or 4% was used in most experiments. At extremely high gelatin concentration, such as 100 g. per 100 ml. solution, a different phenomenon might be expected as reported by Doi and Nikuni (31) and this is considered to be out of the scope of this experiment.

Mixing proportions of starch-chloral hydrate vs gelatin were 1:1, 1:2, 1:3 and 2:1, 3:1, etc.

Various salts were used in order to induce coacervate from the mixture, i.e., a 1% solution of NaCl, KCl, $NaHCO_3$, Na_2SO_4, Na_2HPO_4 or Na_3PO_4.

Dilute KI-I_2 solution or 0.1% solutions of Congo Red or Safranin-O were employed for staining coacervates in order to facilitate the micro-

scopic examination.

Coacervation was carried out at different temperatures, ranging from room temperature to 40°C. It was found that the stability of a coacervate was greatly decreased by increasing the temperature. Therefore the experiment was carried out mostly at room temperature. The pH of gelatin was adjusted to 3, 5, 7 or 8, by addition of 1N HCl or NaOH solution.

RESULTS AND DISCUSSION

Coacervation of Starch by the Action of Chloral Hydrate

When 0.1 to 1.0 g., in 0.1 g. steps, of defatted starch, amylomaize or amylopectin was mixed and heated in a boiling water bath with 10 ml. of 6 M chloral hydrate solution in a test tube, a clear, viscous liquid was obtained within 30 minutes. This was also achieved without heating the mixture. The resulting liquid, upon prolonged standing at room temperature, did not acquire any cloudiness or appearance of containing a solid in suspension. This was confirmed by microscopic examination.

Amylomaize which contained 56-57% amylose was not gelatinized by boiling its water suspension under atmospheric pressure. However the amylomaize was apparently completely dissolved by 6 M chloral hydrate solution with or without heating.

A solution of more than 5% defatted starch, amylomaize or amylopectin was found to be extremely viscous and to tend to solidify upon cooling. Therefore usually a 3-4% concentration was employed.

When a liquid system of 4% defatted starch in chloral hydrate was diluted with water at the ratio of 1:10 at pH 3.5 (original pH of 6 M chloral hydrate aqueous solution), a white cloudiness immediately occurred but rapidly disappeared into solution within a minute. The microscopic observation indicated that the cloudiness resulted from the formation of a large amount of tiny coacervate droplets which were nearly transparent and varied in their size. It was found that the coacervate tends to be more stable at lower dilution ratios or when higher starch concentration

is employed. In this instance, however, the coacervate rapidly disappeared when it was placed on the microscopic slide. This was because of the extremely unstable characteristic of the coacervate that might be attributed to what was described by Bungenberg de Jong as a wetting phenomenon similar to that often seen in certain hydrophilic colloids (20).

After this period, upon prolonged standing at room temperature, a firm, compact white precipitate appeared and gradually increased in quantity during the first few hours. The precipitate then settled down onto the bottom of the container, thus two layers distinctly appeared (Fig. 1). With extremely small dilution ratios, such as 1:1 or less, none or very few needle-shape crystals were obtained. At high dilution ratios, such as 1:20 or greater, however, the separation of two layers was achieved as well. The white precipitate which was described by other workers (42,60) appeared under the microscope as tiny, transparent bilobate particles, sometimes with irregularly shaped outlines, which gradually dissolved upon boiling with the supernatent liquid (Fig. 2).

The shape of the precipitated particles was varied depending on the experimental conditions. At near 3-4% starch or amylomaize in 6 M chloral hydrate solution with the dilution ratio of 1:8, the precipitate was composed of bilobate particles which showed some elasticity when pressed under the cover glass during microscopic examination. At a relatively low concentration of chloral hydrate solution (20-40%) or at a higher dilution ratio (1:20 or more), the precipitate was a mass of single needles or an agglomerate that appeared to be made up of loose sheaf-like bundles of needle-shaped crystals. The precipitate was weakly

Fig. 1. Precipitate of amylose-chloral hydrate complex formed after dilution of a 4% amylomaize (A), 4% defatted starch (B) or 4% amylopectin (C) in 6M chloral hydrate with 10 parts of distilled water.

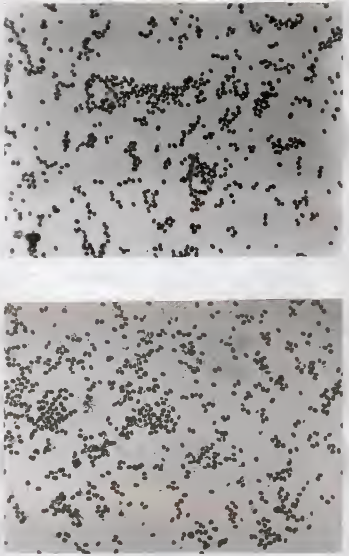

Fig. 2. Microscopic appearance of KI-I_2 stained precipitate of Fig. 1, A (upper) and B (lower) are the corresponding test tube on Fig. 1. Mag. 136X

birefringent (Fig. 3) and stained blue upon addition of $KI\text{-}I_2$ solution, whereas the supernatant liquid stained light violet. It was postulated that the precipitate was an amylose-chloral hydrate complex with a large amount of occluded water in its loose texture.

The amylose-chloral hydrate complex occurred at all concentrations of starch or amylomaize at pH range of 2.5 to 8, but not with amylopectin, so far as was tested. With a fixed concentration of starch (4%) and constant dilution ratio (1:8), the amount of complex was nearly constant on changing chloral hydrate concentration and pH (Table 1).

However the maximum volume of the complex was obtained at a dilution ratio of 1:5 to 1:10 when 4% of starch or amylomaize in 6 M chloral hydrate solution was employed (Table 2).

At fixed concentration of chloral hydrate (6 M) and the dilution ratio 1:8, the amount of complex was increased as the concentration of starch or amylomaize was increased (Table 3). The pH was again found to play no role in the formation of the complex.

A temperature of over $50^{\circ}C$ distinctly reduced the amounts of complex. Prolonged boiling led to the nearly complete disappearance of the complex, but it rapidly reappeared upon cooling. When the complex was placed at room temperature for several weeks or longer, however, it only partially dissolved in the supernatant liquid upon boiling.

Under the same experimental conditions, amylomaize produced the largest amount of complex, followed by defatted starch, and a very low amount was obtained from amylopectin (See Fig. 1). From the fact that the commercially produced amylopectin contained up to 10% amylose, it was thought that the precipitate found in the amylopectin preparation

Table 1. Effect of chloral hydrate solutions on formation of precipitate from 4% defatted wheat starch. Precipitates which formed by dilution with water (1:8) were centrifuged after standing for 24 hours.

Conc. of chl.hyd.%	10	20	30	40	50	70	100(6M)
% ppt. at pH 4.5	41.5	40.0	40.0	39.0	38.9	39.0	39.1
% ppt. at pH 8	40.0	40.0	41.0	38.5	39.0	39.0	38.7

Table 2. Effect of dilution of 4% defatted wheat starch in 6 M chloral hydrate solution with water on formation of precipitate. (pH of water was 3.5).

Dil. ratios	1:3	1:4	1:5	1:6	1:7	1:8	1:9	1:10	1:12	1:16	1:20
% ppt.	32.1	34.5	36.0	37.5	37.5	38.0	36.5	36.2	30.0	28.2	12.7

Table 3. Effect of concentration of starch on formation of precipitate. Starch was dissolved in 6 M chloral hydrate solution and diluted with water by 1:8, at pH 3.5.

Conc. of starch,%	0.5	1.0	1.5	2.0	3.0	4.0
% ppt.	5.0	11.0	16.4	20.1	29.5	38.2

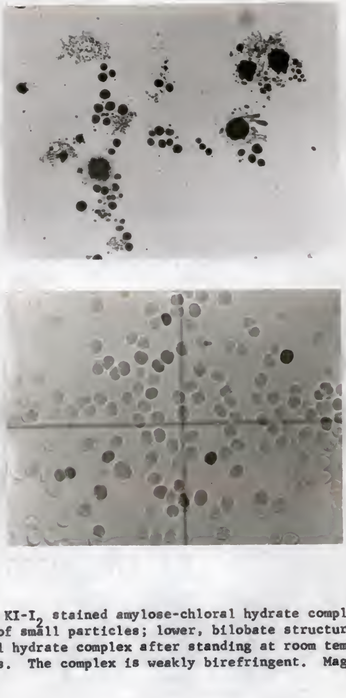

Fig. 3. Upper, $KI-I_2$ stained amylose-chloral hydrate complex, an agglomerate of small particles; lower, bilobate structure of amylose-chloral hydrate complex after standing at room temperature for 3 weeks. The complex is weakly birefringent. Mag. 198X.

might also be an amylose-chloral hydrate complex. Microscopic examination and the reversibility of the precipitate upon heating were found to be similar to these properties of the complex produced from starch or amylomaize preparation.

From the above experiment, it was obvious that a very unstable coacervation occurred at the first stage of dilution, then it rapidly disappeared, followed by a second stage formation of amylose-chloral hydrate complex. However, it is unknown whether coacervation precedes the final complex formation. If it does, then the complex might be expected to include amylopectin as well as amylose since the coacervate should have included both amylose and amylopectin. On the other hand, amylopectin could be lost into the equilibrium liquid as crystals of the complex formed. From the observations made it is impossible to conclude definitely that coacervation does or does not immediately precede complex formation.

Yu (60) stated that the coacervation of starch by the action of chloral hydrate occurred within a definitely narrow range of pH (6.0-7.0) when 50 ml. of 1% starch paste was mixed with 50 ml. of 16% chloral hydrate solution. The action of chloral hydrate was described as a partial dehydration in this instance. However the shape of the coacervate droplets which were shown by Yu was quite similar to that of the amylose-chloral hydrate complex obtained in this experiment.

In the coacervation of an ionized colloid produced by desolvation, either by the action of electrolytes or other means, the surface potential of the solvation layer which surrounding the colloid particle is lowered and the colloid therefore loses stability. To accommodate the

the change of the surface tension -- though it is known to be very small -- the surface of the coacervate usually takes a smooth, round or nearly round droplet form. The droplets do not exhibit crystallinity and are not birefringent. Therefore weakly-birefringent, rough-edged, needle-shaped crystals and bilobate bodies composed of clusters of them which the amylose-complex forms seem hardly to be a coacervate.

The amylose complex which was obtained in this experiment was fairly stable upon addition of salts. This disagreed with the general instability of a coacervate upon addition of excess electrolytes. Moreover coacervation largely depends on the pH and mixing proportions of ingredients, therefore coacervation generally takes place within a specific range of experimental conditions in which all constituents are mixed in such a proportion that coacervation will be favored, as described by Bungenberg de Jong (23). This also suggests a possible difference between coacervation and the amylose complex.

MacMasters _et al_ (42) indicated the evidence of similarity on the basis of an X-ray pattern of amylose-nitroparaffin complex and amylose-chloral hydrate complex which they obtained as a precipitate of disc- and dumbbell-shaped particles about 10 to 20 μ in diameter or length. They further indicated that the complex contained about 23 to 30% chlorine, which decreased to 17% after 9 days. Removal of chloral hydrate from the complex by alcohol extraction resulted in formation of a colorless friable solid which, after drying, sorbed 159 mg. iodine per g. dry wt. If it is assumed that pure amylose sorbs 200 mg. iodine per g., the amylose content of the complex may be considered to be about 80%. This indicates that chloral hydrate may be a good fractionating

agent for amylose.

Although formation of the complex is not a case of coacervation, considerable attention has been given to the phenomenon for two reasons: to distinguish between it and coacervation and to add to the existing concept of the formation of amylose complexes.

Coacervation of Starch by Action of Chloral Hydrate and Gelatin

Four percent of defatted wheat starch, amylomaize or amylopectin was dissolved in 6 M (approximately 100 w/v %) chloral hydrate solution by either heating it in a boiling water bath for 30 minutes or placing it at room temperature for 24 hours without heating. The resulting colorless, viscous liquid was then mixed with 4% gelatin solution at pH 2.5-8.0 at various mixing ratios. The results are shown in Table 4.

Coacervation occurred immediately after mixing at a ratio of 1:1 at pH 3.0-4.0. At the ratios of 1:2 (pH 3.0-4.5) and 1:3 (pH 3.0-4.0) the coacervate occurred only after a very dilute solution of neutral salts was added, i.e., NaCl, Na_2SO_4 etc. (This will be discussed in a later section). With other ratios, coacervation seemed hard to obtain. At pH below 3 or above 5, no coacervation took place. For the best result, a mixing ratio of 1:1 and pH 3.5 were employed.

The coacervate as it occurred appeared as a faint cloudiness which, after standing over night, tended to separate into two distinct layers (Fig. 4). The upper layer, which was equivalent to about 2/5 of the total volume, was colorless and clear. It was colored light violet by diluted $KI\text{-}I_2$ solution and microscopic examination did not show any indication of the presence of particles. The bottom layer, which consisted

Table 4. Effect of pH and mixing ratio on formation of starch-chloral hydrate-gelatin coacervate.

	Mix. Ratio	(4% Starch-chloral hydrate : 4% gelatin)			
pH	1:1	1:2	1:3	2:1	3:1
2.5	-	-	-	-	-
3.0	+	(+)	(+)	-	-
3.5	+	(+)	(+)	-	-
4.0	+	(+)	(+)	-	-
4.5	+ ?	(+)	-	-	-
5.0	-	-	-	-	-
6.0	-	-	-	-	-
7.0	-	-	-	-	-
8.0	-	-	-	-	-

Note: +, First, cloudiness occurred, then visible layers separated out after standing over night.

-, No change was observed

(+), Separation of two layers occurred only after diluted solution of neutral salt was added.

Fig. 4. Coacervate (Bottom Layer) formed by mixing 4% starch in 6 M chloral hydrate with equal amount of 4% gelatin solution at pH 3.5.

of about 2/5 of the total volume, was slightly yellow in color and faintly cloudy. The turbidity was due to the formation of coacervate droplets which were fluid and of large dimension (Figs. 5,6). They were stained blue by dilute KI-I_2 solution and bright orange by Safranin-O solution. This indicated the presence of gelatin in the droplet together with starch components. However the coacervate droplets tended to disappear after about half an hour whereas two layers still remained separated.

Either one of the components, starch, amylomaize, amylopectin or chloral hydrate alone with gelatin solution did not give any cloudiness or droplets. However it was found that a dilute solution of starch paste made by autoclaving 1-3% starch-water suspension at 15 lbs. pressure, (250° F for 1 hour), when mixed with equal amount or more of 3% gelatin solution produced a precipitate on the bottom of the container after standing for 24 hours or longer at 35°C in a thermostat. The volume of precipitate was measured by centrifuging the mixture in a graduated centrifuge tube at approximately 1029 x g. for 10 minutes (Table 5). The precipitate was white; it stained light violet with KI-I_2 solution and was insoluble in water. Microscopic examination indicated that it was chiefly constituted from starch granule sacs.

From the data in Table 5, it might be concluded that the amount of precipitate was nearly proportional to the amount of starch in the mixture. Gelatin, in this instance, seemed to play no role in formation of the precipitate.

It is more likely that the precipitation is merely a retrogradation of starch paste in which the linear fraction of starch aggregates to

Fig. 5. Upper, coacervate droplets obtained after dilution of 4% starch in chloral hydrate and 4% gelatin (1:1) at pH 3.5 with an equal amount of water; lower, same as upper but coacervate was warmed to $40^{\circ}C$ for one minute. KI-I_2 stained. Mag. 198X

Fig. 6. Upper, coacervate droplets formed by mixing 4% amylomaize in 6 M chloral hydrate and 4% gelatin solution (1:1); lower, coacervate droplets of amylopectin-gelatin by same procedure as described above. Mag. 136X.

Table 5. The volume of precipitate produced by mixing various concentrations and amounts of gelatin and starch solution. (3G = 3% gelatin, 1S = 1% starch and so on.)

Mixing propn.	ppt. (ml.)
10 ml, 1S + 10 ml, 3 G	2.3
5 ml, 1S + 5 ml, 3 G	2.3
10 ml, 1S + 10 ml, H_2O	2.1
10 ml, 1S + 5 ml, H_2O	2.0
5 ml, 1S + 10 ml, H_2O	0.8
10 ml, 3S + 10 ml, 3 G	6.6
10 ml, 3S + 5 ml, 3 G	6.5
10 ml, 3S + 10 ml, H_2O	6.3

NOTE: Starch solution made by autoclaving starch-water suspension at 15 lbs. pressure, 250° F for 1 hour.

form an insoluble precipitate as the paste is being aged. With higher gelatin concentration, the precipitate tends to resist settling when it is centrifuged, probably due to the fact that the gelatin occludes the starch granule sacs to form a viscous, elastic layer around each individual granuler sac, thus indirectly increasing the volume of precipitate. This was evidenced by the fact that at higher gelatin concentration, the volume of precipitate was slightly more than at lower gelatin concentration at the same starch level, as seen in Table 4.

Ostwald and Hertel (49) reported upon the precipitate produced by mixing wheat starch paste and gelatin solution. With constant gelatin concentration (4%), the amount of precipitate proportionally increased as the concentration of starch (0.25-2.0%) increased. With constant starch concentration (2%) and variable gelatin concentration (1-8%), the volume of precipitate was nearly constant, except for a very small peak at a mid-range of gelatin concentration. The precipitate was reported to be a white, voluminous or compact mass depending on the level of gelatin concentration. Neither the formation of new phases nor, more specifically, of coacervate droplets was described in this instance. The precipitate which Ostwald obtained by centrifuging the mixture either immediately or 10 minutes after mixing starch and gelatin might be composed of granule sacs or of retrogradated amylose.

It was pointed out by Koets (40) that in neutral or slightly acid solution, the negative charge of amylose is too small to bring about coacervation with positively charged proteins; the mutual attraction being too small to overcome the repelling force of the micelle hydration. He further demonstrated that starch was capable of coacervation

by changing the hydroxyl groups of amylose to a strongly negative group. Thus he obtained a starch coacervate by mixing amylophosphoric acid and gelatin at pH 5, in which case a sufficiently high density of charge of amylophosphoric acid was now able to overcome the repelling force of the micelle hydration.

The coacervate which was obtained by mixing starch-chloral hydrate solution and gelatin in this experiment was so unstable that it disappeared into solution within about 30 minutes. It was first assumed that when starch is dissolved in chloral hydrate, a new breed of micelle structure, which is independently related to linear or branched fraction, might be expected in which the density of charge would become much higher than for starch alone. Finar (34) pointed out that chloral hydrate in aqueous solution is very acidic and that it has hydrogen bonds between two hydroxyl groups and two chloride ions. This suggests a possible mode of formation of a "starch-chloral hydrate mixture" as a hydrophilic colloid with an appreciable density of charge and with the consequent ability to combine with positively charged colloidal protein to form coacervate droplets. If the production of a coacervate of a hydrophilic colloid depends on the removal of stabilizing factors (capillary charge of micelle and solvation water surrounding the micelle); the action of gelatin on starch-chloral hydrate complex might be explained as a dedolvation such as has been studied upon coacervation of gelatin and gum arabic by many workers.

Bungenberg de Jong and Kruyt (4,5) suggested that the tendency of a hydrophilic colloid to enter into a coacervate is dependent on the density of charge on the surface of the colloid micelle. Koets (40)

further indicated that the better the opposite charges are balanced and the greater the difference in charge, the greater will be the mutual dehydration. Thus a coacervate may separate out in a relatively unstable, liquid-rich form (high water content of coacervate droplet) or a more stable, compact and plastic mass, depending on the charge characteristics of an individual colloid.

From the view described above, it was postulated that the micelle of "starch-chloral hydrate mixture" possesses a relatively low density of charge; the charge is so little that it is just enough to overcome the repelling force of the water of hydration that is surrounding the micelle, in order to permit it to approach the gelatin molecule. In this instance the starch-chloral hydrate micelle might unite with a gelatin micelle, together with a small but decreased amount of hydration water. The coacervate in this instance should be voluminous, rich in equilibrium liquid, and therefore unstable. This is a complex coacervation in which an adequate charge opposition between two colloids causes coacervation. As previously described, the coacervation of starch-chloral hydrate and gelatin occurred at pH 3.0-4.0. At a pH higher than that of the isoelectric point (4.8), gelatin exhibits negative charges, therefore no coacervation might be expected. Since the charge effect of starch-chloral hydrate mixture is weak, the lower the pH of the gelatin, the greater the difference in charge (greater effective attraction between the particles) will be. Thus a coacervate is most easily produced at pH 3.0-4.0. At extremely low pH, another factor is encountered. That is a resultant high electrolyte concentration (considerable amount of acid must be added to bring the pH down) and possible

changes in protein nature. This may also be unfavorable for coacervation to take place.

The Action of Water and Salts

It has previously been described that an instability of the coacervate which was produced by the action of gelatin on starch-chloral hydrate mixture was chiefly due to the insufficient charge effect of the micelles of the starch-chloral hydrate mixture, i.e., the density of charge was not large enough to expell all hydration water which was surrounding the micelle bodies. Therefore the resulting coacervate droplets tended to include more hydration water in their make-up.

Upon addition of a limited amount of water (pH 6.8) or of 0.01% salt solution (NaCl, Na_2SO_4, Na_3PO_4 etc.) to either fresh starch-chloral hydrate-gelatin mixture or to the mixture after the unstable coacervate had disappeared, a large number of coacervate droplets were produced. The system, originally nearly transparent, was now highly turbid. The coacervate as it was seen under the microscope was composed of spherical droplets of various sizes; some were composite coacervate drops, each consisting of from two to more coexisting droplets (Figs. 7,8,9).

An attempt was made to establish a quantitative relationship between the composition of the coacervate and that of the equilibrium liquid at various salt concentrations; however it failed, chiefly because the isolation of the coacervate was found to be extremely difficult, since the coacervate has the same constituents as occur in the equilibrium liquid. Turbidity could not be used as a measure of the amount of coacervate, since the system was not homogeneous; the droplets

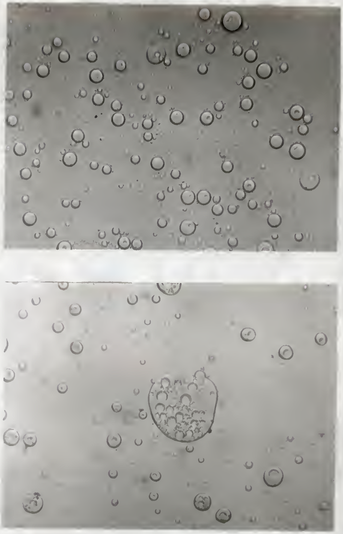

Fig. 7. Complex coacervate of starch-chloral hydrate gelatin formed by dilution of a mixture (pH 3.5) of 4% starch in 6 M chloral hydrate and 4% gelatin (1:1) with an equal amount of distilled water. Upper, at room temperature; lower, the coacervate was warmed to 40°C for 1 minute, then gradually cooled to room temperature. Originally non-vacuolized or slightly vacuolized coacervate droplets were now strongly vacuolized. A typical composite coacervate was formed. Mag. 198X

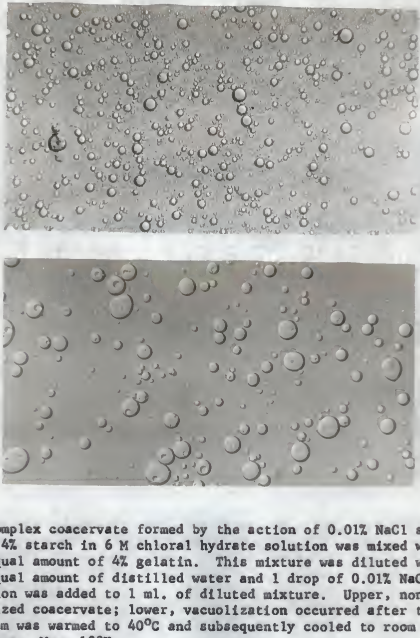

Fig. 8. Complex coacervate formed by the action of 0.01% NaCl solution. A 4% starch in 6 M chloral hydrate solution was mixed with an equal amount of 4% gelatin. This mixture was diluted with an equal amount of distilled water and 1 drop of 0.01% NaCl solution was added to 1 ml. of diluted mixture. Upper, non-vacuolized coacervate; lower, vacuolization occurred after the system was warmed to 40°C and subsequently cooled to room temperature. Mag. 198X

Fig. 9. Complex coacervate of starch-chloral hydrate gelatin formed by the action of Na_2SO_4 (upper) or Na_3PO_4 (lower). One drop of 0.01% Na_2SO_4 or Na_3PO_4 solution was added to the mixture of 4% starch in 6 M chloral hydrate and 4% gelatin. Mag. 198X

might settle down to the bottom of the container as it was being placed in the electrophotometer. Furthermore the size of the droplets was found to be variable depending on the concentration of salt used.

The droplets, however, exhibited the general characteristics of a coacervate (such as reversibility, vacuolation, etc.) which have been described in the literature. The fact that the droplets stained blue by diluted $KI\text{-}I_2$ solution or stained bright orange by Congo Red or Safranin-O solution indicated that both starch and gelatin were present in them. The coacervate was reversible. When it was diluted with a large excess of water (for example, 3 drops of water to one drop of coacervate), the coacervate rapidly disappeared. However the coacervates were again produced upon removal of excess water without heating. This was done by using an electric fan to send forced air over the coacervate on the microscopic slide glass. The droplets thus produced were the same in appearance as the original coacervates. The sequence was observed by use of the microscope (Fig. 10).

Upon heating, the coacervate droplets gradually became indistinct aroung their edges, seemed to become confluent with the surrounding liquid and finally disappeared at 70°C. Rapid cooling of the remaining solution brought about reappearance of the coacervate. But in this second appearance the number of droplets seemed to be fewer.

Defatted wheat starch, amylomaize and amylopectin all produced coacervates that behaved the same under the conditions described above (Fig. 6).

Production of a coacervate upon dilution with water was long ago observed by Bungenberg de Jong (5,22). It was suggested as one of the

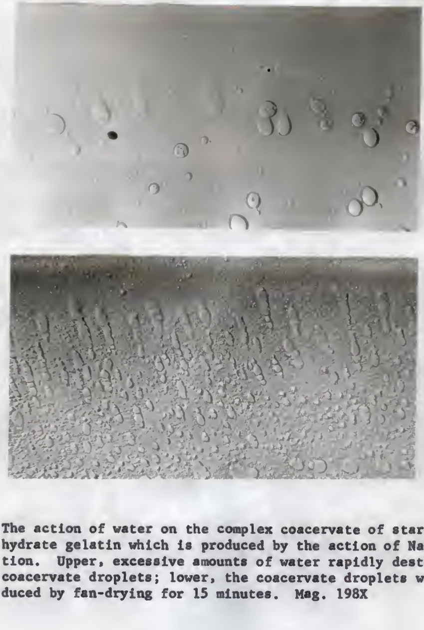

Fig. 10. The action of water on the complex coacervate of starch-chloral hydrate gelatin which is produced by the action of NaCl solution. Upper, excessive amounts of water rapidly destroyed the coacervate droplets; lower, the coacervate droplets were reproduced by fan-drying for 15 minutes. Mag. 198X

characteristic phenomena of the production of complex coacervates from hydrophilic colloids. Water in this instance, as was explained, acts as a weak electrolyte that interferes with the equilibrium among micelles, i.e., to diminish the repulsive force and to increase the effective attraction between two colloidal particles.

However, the present work has shown that while the amount of water added is critical for the production of a coacervate, the stage at which it is added is equally critical. Bungenberg de Jong's suggestion does not explain this. It is more likely that the water affects the "partial miscibility" of hydrophilic colloids in an unexplained way to diminish the water content of the colloidal micelles. In either case, the amount of water added is a critical factor; that is, a little excess in the amount of water might affect the proper mixing proportion of two colloids. This, apparently, would not induce coacervation.

Coacervation of a hydrophilic sol generally demands the removal of two stability factors: electrostatic charge of the colloidal micelle and dehydration. Present work showed that the starch-chloral hydrate mixture formed a coacervate with gelatin probably by mutual dehydration of these two oppositely charged colloids. This is more definitely shown by addition of a limited amount of a neutral salt to the mixture of starch-chloral hydrate and gelatin. When 1-3 drops of 0.01% NaCl, Na_2SO_4 or Na_3PO_4 solution was added to 1 ml. of the above mixture, a thick and white cloudiness immediately occurred. When a salt was added without disturbing the sol, the coacervate formed a thick, white layer on top of the solution (Fig. 11). Microscopic examination showed the production of a large number of spherical droplets of coacervate which contained

Fig. 11. Salt solution was added to the mixture of 4% starch-chloral hydrate and 4% gelatin (1:1) without disturbing the mixture. The coacervate was formed immediately after addition as a thick, white layer.

vacuoles. The droplets were stained blue by diluted $KI-I_2$ solution, and bright orange by Safranin-O solution, like the coacervate obtained with dilution by water. The coacervate was very stable; after standing 3 days at room temperature, the droplets still remained unchanged.

As they are electrolytes, the salts when added to the oppositely charged hydrophilic colloids, diminish the repulsive force which opposes the effective attraction of colloidal micelles and thus allow the indifferent colloidal micelles to approach each other. Assume that the action of a salt were just sufficient to diminish the repulsive force of the water of hydration around the micelles and thus permit two oppositely charged colloidal micelles to come into contact and to fuse together, finally to form a larger molecular skein; this would cause immediate production of a coacervate. The more the repulsive force is diminished, the lesser the water content of the coacervate will be. The coacervate in this instance would contain less water, therefore it would be denser and more stable as compared with that produced without salt effect. In fact, the usefulness of a neutral salt as an effective desolvation agent has often been demonstrated by Bungenberg de Jong et al (5,8,11,37).

However, the remarkable effect of salts may be useless when an excessive amount is used. It was found that when 1 drop of 5% NaCl (or Na_2SO_4, Na_3PO_4) solution was added to 1 ml. of a coacervate of starch-chloral hydrate and gelatin which was produced by addition of 1-3 drops of 0.01% NaCl, the coacervate which originally existed was immediately dispersed. The salt in this instance, not only diminished the repulsive force of the water of hydration but also evidently

diminished the charge of the colloidal micelles and this apparently inhibited the production of a coacervate. High salt concentration may also change the protein nature of gelatin; presumably this would also inhibit the production of a coacervate.

Among the many characteristic phenomena of complex coacervation so far as reported, vacuolation and dye adsorbing properties are unique. Vacuolation was found in many coacervates which were obtained in this work (See Figs. 5,7,8). A vacuole, as Bungenberg de Jong (20) describes it, is an inclusion of equilibrium liquid in the form of fine drops throughout the whole concervate. It is produced when the equilibrium between coacervate and equilibrium liquid is changed through some variable. The equilibrium between the coacervate and the equilibrium liquid depends on the temperature. When temperature is high the equilibrium moves toward the direction in which more solvating liquid is included in the coacervate, according to Bungenberg de Jong. Further rise of temperature would lead the coacervate to contain more and more solvating liquid and finally the coacervate droplet would fuse into the liquid and disappear. In this way the instability of a coacervate is explained in term of solubility.

Now, if careful increase in temperature is conducted to such a point that it would not break the droplet (35°C, in present work) and then the system is cooled slowly to room temperature, the liquid will gradually diffuse out of the coacervate (because of the change in equilibrium), without destroying the homogeneity of the coacervate droplet. The equilibrium liquid which diffuses out to one spot in a coacervate droplet thus forms a vacuole inclusion. It may be one large single

droplet or relatively small multiple vacuoles, depending on the nature of the coacervate, the temperature and the rate of cooling. Therefore a vacuole is essentially the equilibrium liquid included in a coacervate droplet. It is fairly stable if the temperature is unchanged after it has once formed.

A different type of vacuole may also be produced by slight agitation of the starch-chloral hydrate mixture while adding the gelatin. In this instance the vacuole will be an air inclusion and the temperature does not affect its production or nature. The vacuoles produced in this instance were also fairly stable; they remained for at least 24 hours. These different kinds of vacuoles can be identified by staining with $KI-I_2$, Congo Red or Safranin-O solution, since an air vacuole does not stain with any one of these, whereas the vacuole which is composed of equilibrium liquid stains the same color as the coacervate.

Bungenberg de Jong described other types of vacuolation that occurred without a change in temperature or agitation, merely by modifications in the composition of the equilibrium liquid that caused lowering of solvation. Still others, such as the vacuoles produced upon application of direct current to a complex coacervate, were described.

Bungenberg de Jong described the adsorption by complex coacervates on their surface of different particles such as those of carbon, carmine, and dyes, as well as of particles of biological origin such as pollen, grain, and various mold spores. The coacervate of starch-chloral hydrate and gelatin also showed this phenomenon common to complex coacervates. It was found that a starch-chloral hydrate-gelatin

coacervate (induced by the action of NaCl solution) adsorbed a small amount of Safranin-O dye powder which was added to the system after the coacervate was produced (Fig. 12). Microscopic observation showed that the dye was adsorbed only on the surface of coacervate droplets and occurred not at all in the equilibrium liquid.

It is known that microparticles will be concentrated at the surface between two immiscible liquids when they are mixed and gently shaken. The phenomenon is probably due to the difference in wetting ability of two liquids that causes the adhesion of insoluble microparticles at the interface, in this experiment, at the surface of the coacervate droplet.

The importance of vacuolation and dye absorption properties is not well explained and more extensive observation is necessary before any conclusion can be made; however, the existence of such phenomena provides the evidence that the starch-chloral hydrate-gelatin coacervate is a complex coacervate.

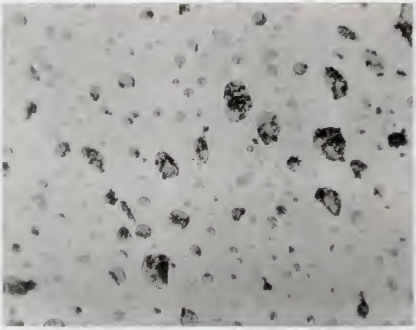

Fig. 12. Dye adsorption of the complex coacervate of starch-chloral hydrate-gelatin. Safranin-O powder was adsorbed only at the interface of two phases, at the surface of the coacervate droplets. Mag. 198X

SUMMARY AND CONCLUSIONS

An unstable starch coacervate was observed upon mixing 3-4% defatted wheat starch in 6 M chloral hydrate solution and diluting the resulting clear, viscous liquid with about 8 times its volume of water at pH 3.5. This coacervate, however, disappeared rapidly when it was placed on a microscope slide. The instability of this coacervate was probably due to the wetting phenomenon which is often seen in certain instances of the coacervation of hydrophilic colloids. When the system was allowed to stand at room temperature, a second precipitation was observed. It was found that this precipitation was an amylose-chloral hydrate complex and that it could be obtained at any of a wide range of starch concentrations, dilution ratios and pH, i.e., 1-10% starch in 10-100 w/v% chloral hydrate solutions at pH 3-8. Amylose preparations produced the most precipitate, followed by wheat starch; and amylopectin preparations produced almost no precipitate. The complex exhibited rather poor crystallinity, but showed well-formed crystals that are not seen in a coacervate. A probable error in identification of amylose-chloral hydrate complex as a starch coacervate due to indirect dehydration was briefly discussed.

During the experiment, amylomaize which contained 56-57% amylose was found to be soluble in 6 M-chloral hydrate solution with or without heating in a water bath.

A starch-gelatin coacervate was obtained when one part of 4% starch, amylomaize or amylopectin in 6 M-chloral hydrate solution was mixed with one part of 4% gelatin solution at pH 3.0-4.0. The coacervate droplets appeared to be spherical in shape and exhibited the general characteristics

of a complex coacervate. They were unstable upon prolonged standing (30 minutes) and completely disappeared upon heating. But the coacervate reappeared on cooling.

When a limited amount of water or of 0.01% salt solution (such as NaCl, Na_2SO_4 and Na_3PO_4) was added to this unstable mixture, a stable coacervate was obtained. It exhibited general characteristics of a complex coacervate, i.e., reversibility, vacuolation, dye adsorption, etc. The mechanism of production of a starch-gelatin coacervate was described to be attraction of weakly charged starch-chloral hydrate micelles (negative) to positively charged gelatin micelles; thus a large conglomerate of two or more oppositely-charged micelles (coacervate) was produced, in terms of mutual dehydration. The degree of dehydration determines the nature of the coacervate, i.e., a coacervate of fluid-rich, unstable mass or a coacervate of rigid, compact, stable spherical droplets. Generally a limited amount of salt acts as a good desolvation agent and therefore stable spherical coacervate droplets can be obtained upon addition of salt. But an excessive amount of salt inhibits or destroys the coacervate because of complete disappearance of micellar charge and possible change in protein nature of gelatin.

Vacuolation and dye adsorption properties of complex coacervate were demonstrated and discussed.

Evidence was shown that starch is capable of forming a coacervate with positively charged gelatin, when the starch is first dissolved in chloral hydrate. It was previously observed that starch produces a colloidal micelle with chloral hydrate and thus, probably, exhibits a certain amount of capillary charge on its micellar surface. Although

the charge is not as large as that of gelatin, however, it is large enough to attract positively charged gelatin micelles and to induce mutual dehydration, thus coacervation takes place.

Salts effectively promoted the dehydration process. The role of salt clearly provided the evidence of what was suggested as the mechanism of coacervation in the present work. Electrophoresis of coacervate droplets might provide a close view of the nature of the coacervate in term of electric charge of the micellar structure. Under a strong A.C. electric field, a complex coacervate droplet would be deformed, and the original spherical droplet would become flat, disc-like or ellipsoidal shape as described by Bungenberg de Jong (20). Future investigation might include studies on electrophoresis of the coacervate described here.

Starch and gelatin evidently both were present in the coacervate droplets. This was shown by staining of the droplets with reagent and dyes such as $KI\text{-}I_2$, Congo Red and Safranin-O solution. However the method of quantitative analysis of coacervate composition should be employed; only this can provide a definite knowledge of the composition of the coacervate at various mixing ratios and pH.

Starch has been reported by earlier workers to be unable to form a coacervate unless the hydroxyl groups are changed to strongly dissociated radicals. This work presents one example of starch coacervation which might involve the formation of colloidal micelle of starch-chloral hydrate mixture, prior to formation of a complex coacervate.

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ABSTRACT

Coacervation is a colloidal phenomenon in which a colloid-rich phase (coacervate) and a colloid-poor phase (equilibrium liquid) are separated from a colloidal system by an action which removes the stabilizing factors of the hydrophilic colloid, i.e., by change in pH, presence of indifferent colloids, presence of electrolytes, etc. A coacervate may be obtained as spherical droplets, myelin structures or large sheets that may be visible under the microscope.

Starch coacervation has been frequently reported, yet the importance of it has received relatively little attention. Starch sponge which is produced by the unique phenomenon of starch coacervation by freezing, however, is one of the most successful applications of coacervation.

This investigation presents an example of starch coacervation which involves the charge effect on a hydrophilic colloidal micelle. Starch paste when mixed with chloral hydrate solution and diluted with water produced only extremely unstable tiny microscopic droplets which upon standing, rapidly disappeared. A few hours later a white precipitate formed and it was postulated to be an insoluble amylose-chloral hydrate complex.

Starch was found to be able to form a coacervate with gelatin when it was dissolved in concentrated chloral hydrate solution prior to mixing with gelatin. Thus starch-gelatin complex coacervation was obtained by dissolving 3-4% starch in 6 M (about 100 w/v%) chloral hydrate solution and mixing one part of this liquid with one part of 4% gelatin at pH 3.0-4.0. Coacervation was promoted by addition of a limited amount of water or 0.01% of neutral salt solutions, such as NaCl, Na_2SO_4 and

Na_3PO_4.

The coacervate occurred as spherical droplets which exhibited the general characteristics of a complex coacervate such as vacuolation, reversibility, dye adsorption etc. The coacervate was produced in a narrow range of pH and mixing proportions. It was found to be fairly stable.

It was postulated that the starch when dissolved in chloral hydrate solution forms an unknown type of colloidal micelle with chloral hydrate, since chloral hydrate is known to possess two active hydroxyl groups which tend to form hydrogen bonds with other groups. It is possible that after formation of a starch-chloral hydrate micelle, this micelle unites with gelatin to form a complex coacervate. The force which leads the micelle and gelatin to unite with one another might be mutual dehydration taking place when negatively charged micelles of starch-chloral hydrate and positively charged gelatin micelles approach each other.

The fact that addition of neutral salts may promote coacervation provides evidence that a charge effect causes starch-chloral hydrate-gelatin complex coacervation, because a limited amount of salt might act to effectively remove the solvation layers which surround the approaching micelles. Therefore more compact, less liquid and stable coacervate droplets are obtainable when a neutral salt is effectively used.